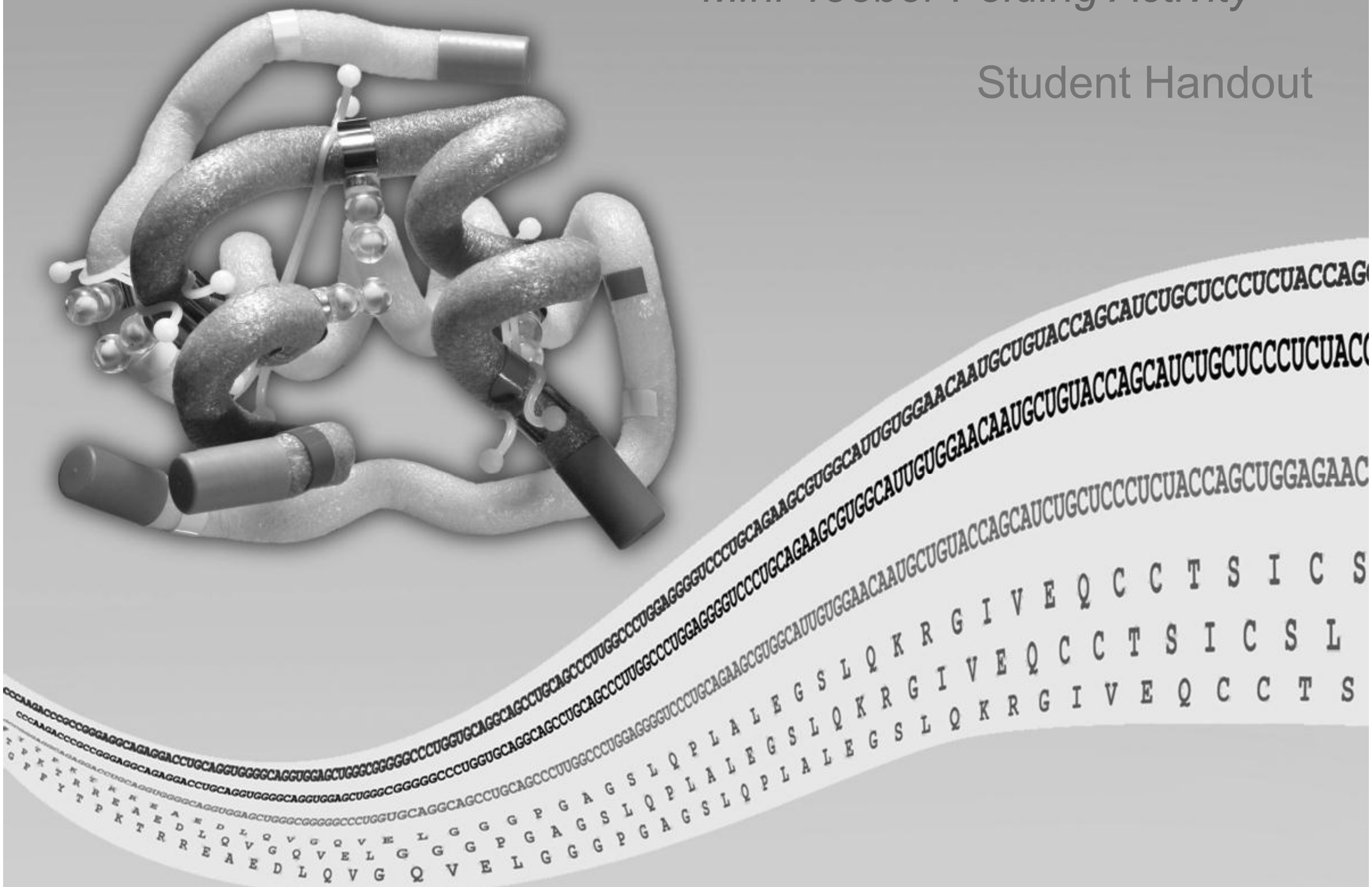
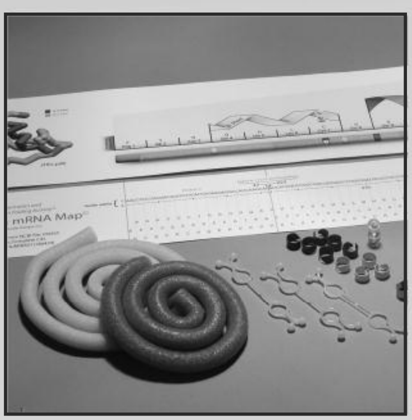
Insulin mRNA to Protein Kit©

A 3DMD Paper BioInformatics and Mini-Toober Folding Activity©

Student Handout





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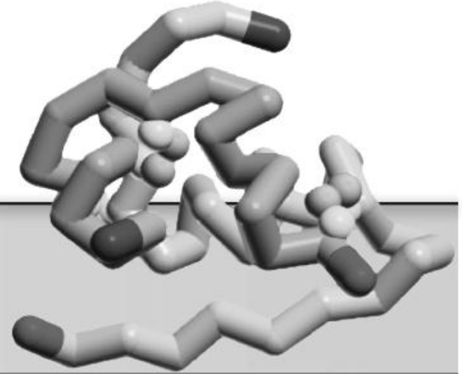


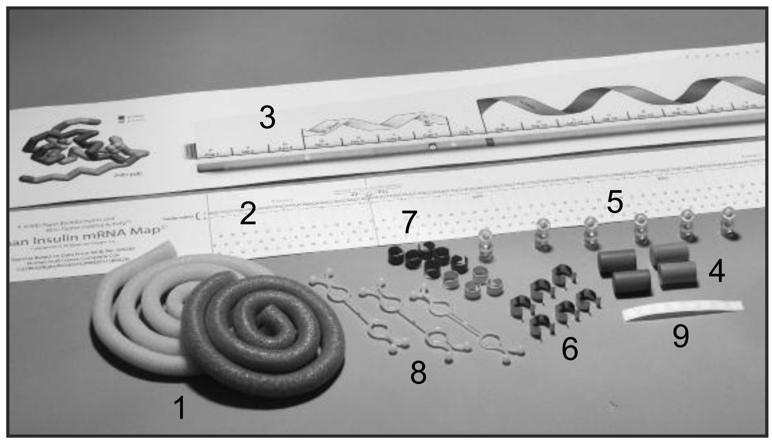
Insulin mRNA to Protein Kit©

Contents

Parts

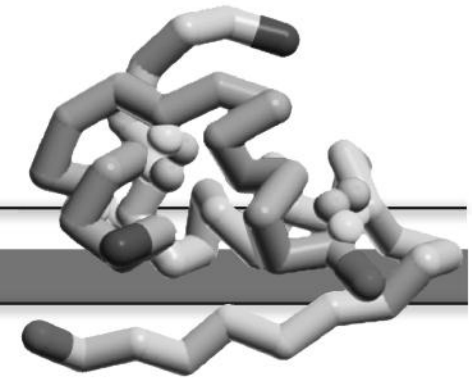
1. Mini-Toobers (orange and purple)
2. Insulin mRNA Map
3. Insulin Mini-Toober Folding Map
4. Endcaps
5. Sidechains
6. Metal Clips
7. Plastic Markers
8. Support Posts
9. White Dots

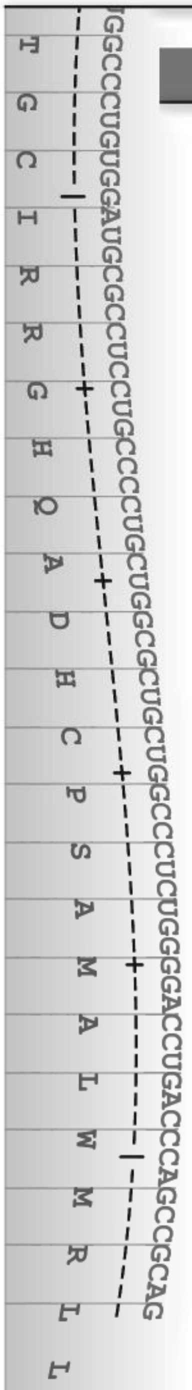
Not shown: CD with animation, background information, teacher notes, Jmols, resources, and other activities and supporting information



Why Is Insulin Important?

Insulin is a protein (peptide hormone) that plays a major role in glucose homeostasis – the regulation of your blood sugar levels. After you eat a meal insulin is normally released into your blood and triggers your liver, muscle, and fat cells to take up glucose from your bloodstream. Once inside these cells, the glucose can be used to fuel the production of ATP (adenosine triphosphate). ATP is frequently called the universal molecular currency because it transfers energy in our cells. *See the animation on the CD included in the kit for more information on the role insulin plays in regulating blood sugar and the uptake of insulin.*



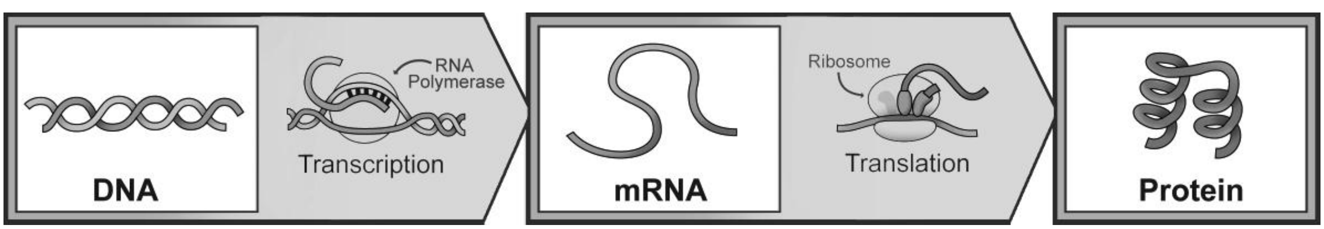


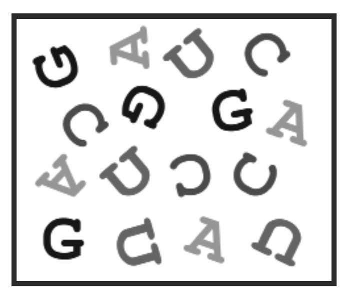
Insulin Paper BioInformatics Activity

In this activity, you will explore the steps involved in the synthesis of the protein, starting with insulin mRNA. Specifically, you will consider how this mRNA is translated by the ribosome into a precursor form of insulin, and then how the precursor is *processed* to create the final, functional protein. As the final step in this activity, you will create a physical model of insulin by folding two Mini-Toobers (foam-covered wires) into the precise 3-D shape of the A-Chain and the B-Chain of this protein.

Becoming Familiar with the Data

A gene encoded within the DNA of a chromosome is transcribed into mRNA in the nucleus of a cell. The mRNA is then transported into the cytoplasm, where a ribosome reads the code and builds a protein (translation). This activity focuses on how the insulin mRNA is translated into the insulin protein.





1. Unroll your Insulin mRNA Map and look at the green-colored sequence of letters at the top of the map.

a. What different letters appear in this sequence?

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b. What do these letters represent?

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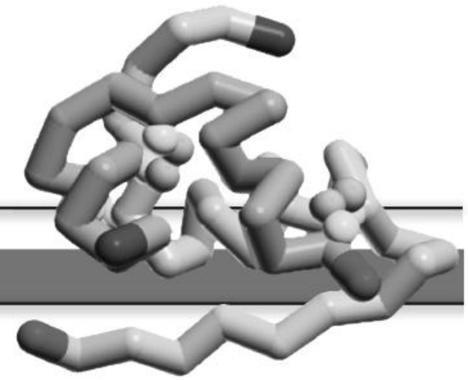


The Standard Genetic Code

When RNA polymerase initially transcribed the insulin gene into messenger RNA, two introns – totaling 966 additional nucleotides – were included in the precursor form of the insulin mRNA. These intron sequences were removed from the mRNA in a splicing reaction as the mRNA was being transported out of the nucleus of the cell. You might want to discuss why almost all eukaryotic genes contain introns.

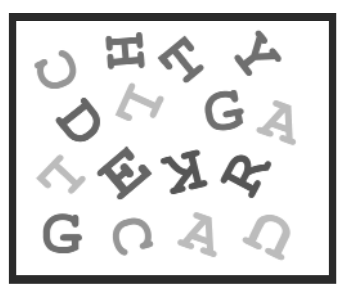
Second Letter

First Letter	U	C	A	G	Third Letter
U	UUU → Phe F	UCU → Ser S	UAU → Tyr Y	UGU → Cys C	U
	UUC → Phe F	UCC → Ser S	UAC → Tyr Y	UGC → Cys C	C
	UUA → Leu L	UCA → Ser S	UAA → Stop	UGA → Stop	A
	UUG → Leu L	UCG → Ser S	UAG → Stop	UGG → Trp W	G
C	CUU → Leu L	CCU → Pro P	CAU → His H	CGU → Arg R	U
	CUC → Leu L	CCC → Pro P	CAC → His H	CGC → Arg R	C
	CUA → Leu L	CCA → Pro P	CAA → Gln Q	CGA → Arg R	A
	CUG → Leu L	CCG → Pro P	CAG → Gln Q	CGG → Arg R	G
A	AUU → Ile I	ACU → Thr T	AAU → Asn N	AGU → Ser S	U
	AUC → Ile I	ACC → Thr T	AAC → Asn N	AGC → Ser S	C
	AUA → Ile I	ACA → Thr T	AAA → Lys K	AGA → Arg R	A
	AUG → Met M	ACG → Thr T	AAG → Lys K	AGG → Arg R	G
G	GUU → Val V	GCU → Ala A	GAU → Asp D	GGU → Gly G	U
	GUC → Val V	GCC → Ala A	GAC → Asp D	GGC → Gly G	C
	GUA → Val V	GCA → Ala A	GAA → Glu E	GGA → Gly G	A
	GUG → Val V	GCG → Ala A	GAG → Glu E	GGG → Gly G	G

- translation start codon
- translation stop codon
- hydrophobic amino acids
- hydrophilic non-charged amino acids
- negatively charged amino acids
- positively charged amino acids
- cysteine

Translation Reading Frames

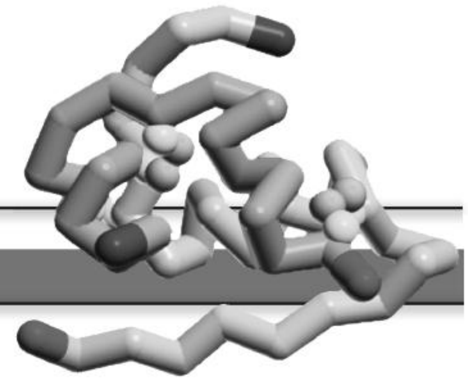
2. Look at the three **blue sequences** at the bottom of the Insulin mRNA map.

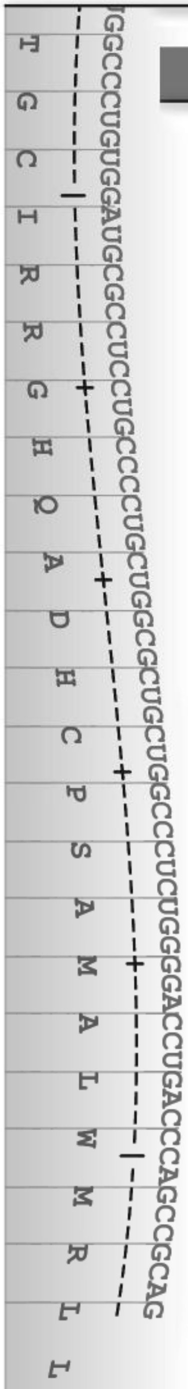


a. What different letters appear in these blue sequences? How many different letters appear in these sequences?

____

____





Translation Reading Frames (continued)

b. What do these letters represent?

c. What is the relationship between the green letters at the top of the strip to the blue letters at the bottom?

d. Why are there three blue sequences?

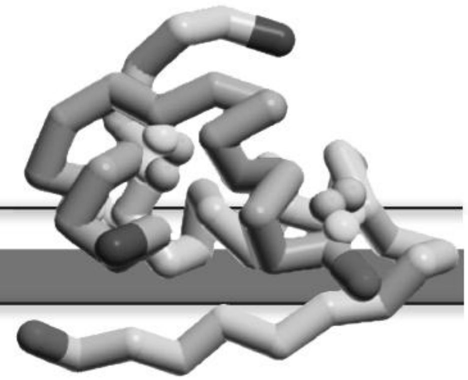
e. What do you think the asterisks (*) represent in the blue sequences?

Identifying the A-Chain and the B-Chain

The insulin protein actually consists of two separate chains, known as the **A-Chain** and the **B-Chain**. The amino acid sequences of the two chains are shown below:

A-Chain
G I V E Q C C T S I C S L Y Q L E N Y C N

B-Chain
F V N Q H L C G S H L V E A L Y L V C G E R G F F Y T P K T



Identifying the A-Chain and the B-Chain (continued)

3. Locate, highlight and label the A-Chain and the B-Chain amino acid sequences on your Insulin mRNA Map.

   a. What do you notice about the location of the A-Chain and B-Chain amino acid sequences within the BioInformatics Map?

   ____

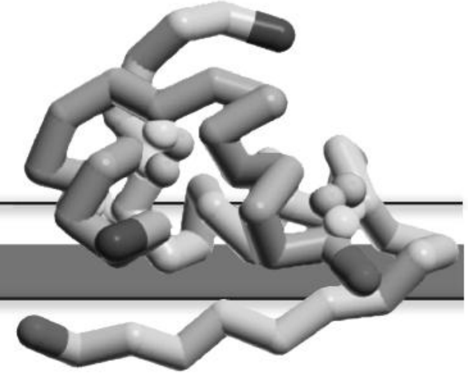
   ____

Note: The subunit composition of insulin (two chains) was known before the sequence of the gene was determined. Unfortunately, when the gene was sequenced, it was discovered that the B-Chain was encoded before the A-Chain – which has been confusing biology students ever since!

Translating mRNA Into Protein

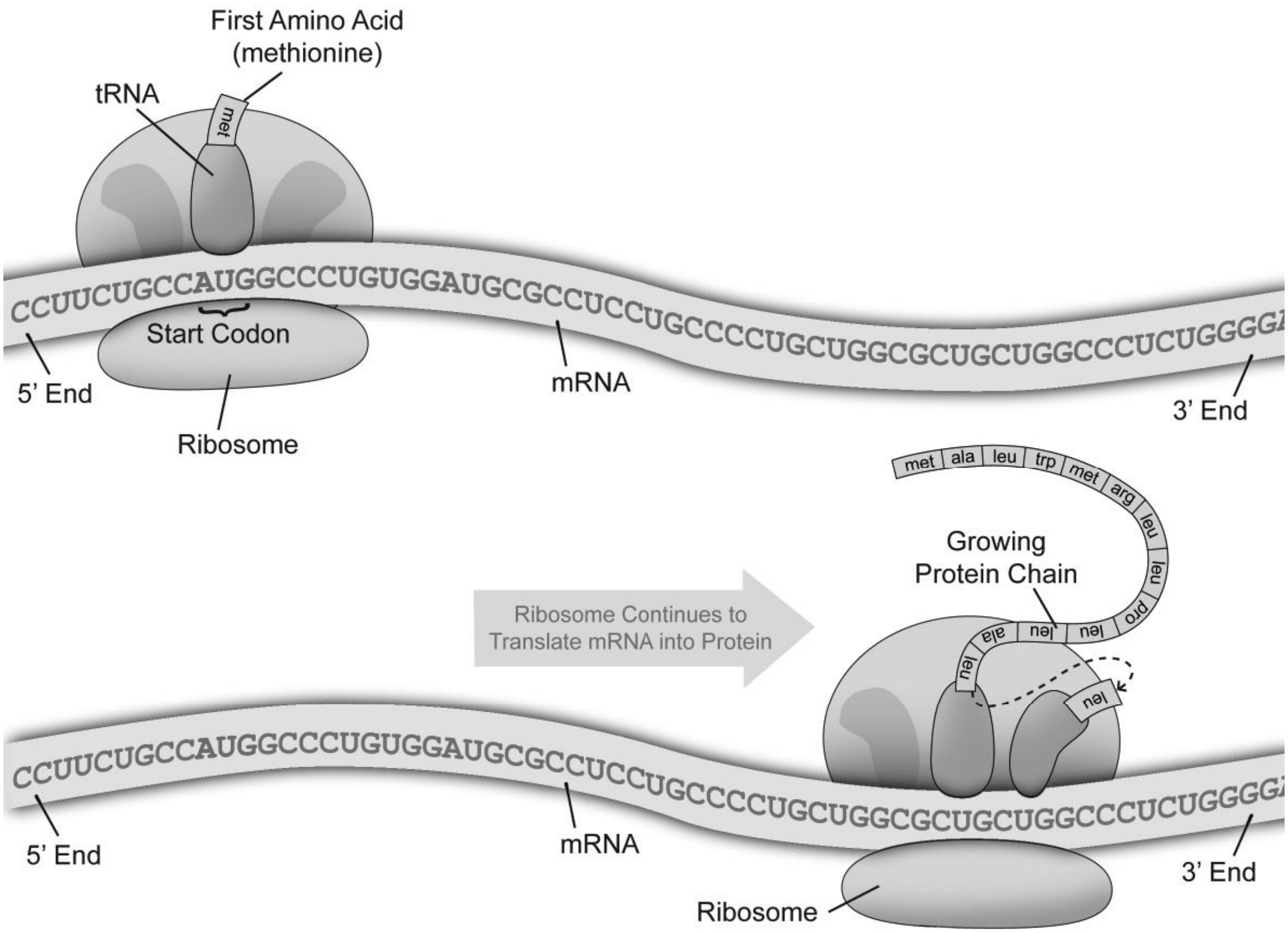
To translate an mRNA into protein, the ribosome recognizes an AUG codon – and begins decoding the mRNA as it moves from left to right (5’ to 3’) down the mRNA sequence. As a result, all proteins begin with the amino acid methionine (Met, M) at their N-terminal end.

In humans and other eukaryotes the ribosome begins synthesizing proteins at the first AUG codon from the 5’ end of the mRNA.



Translating mRNA Into Protein

Protein Synthesis of Insulin Protein



4. Highlight the protein that is synthesized by a ribosome. The ribosome binds to the first AUG located downstream (to the right) of the 5' end of the mRNA to begin synthesis.

a. Where does the protein stop?

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b. How many amino acids are in the insulin protein?

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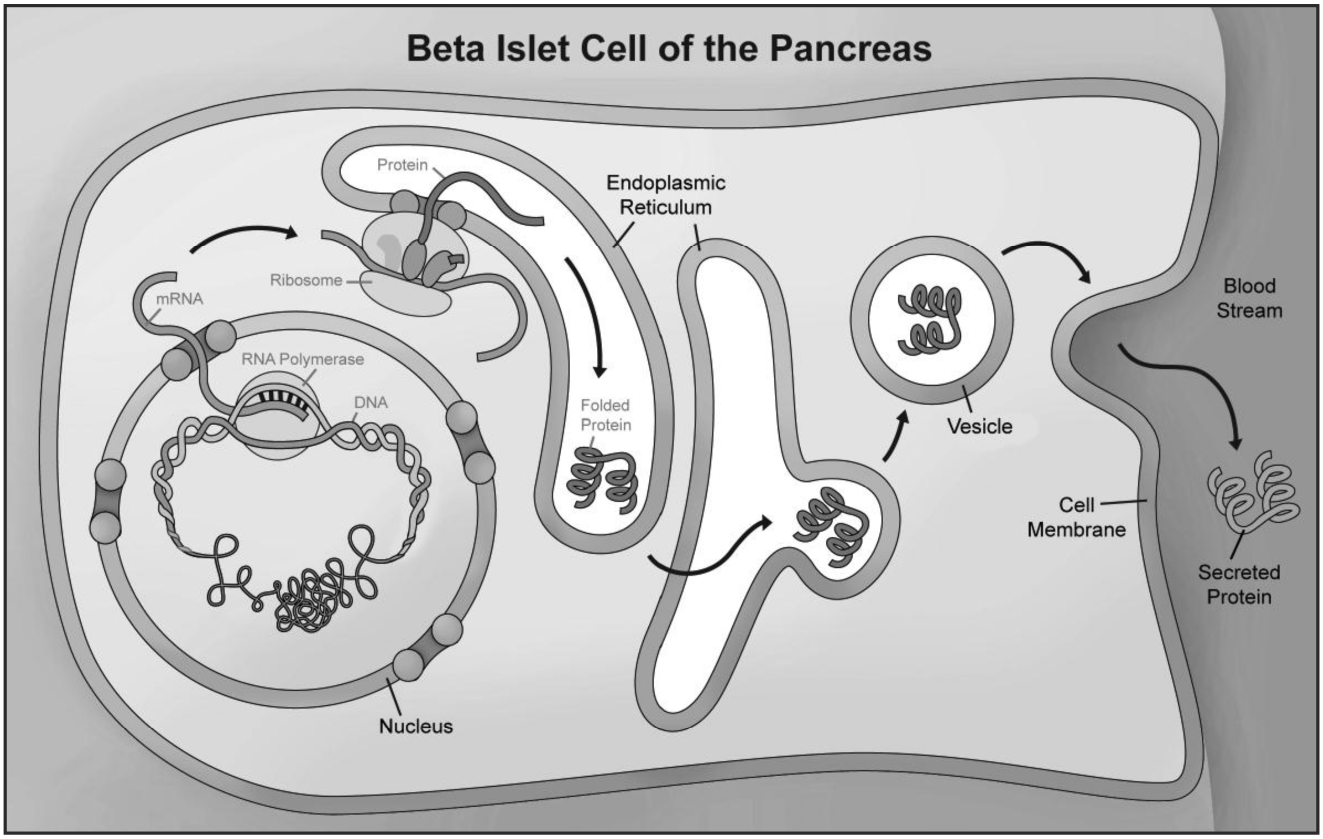
Preproinsulin - the Precursor Form of Insulin



Insulin is synthesized in beta islet cells of the pancreas. Following a meal, it is secreted from these cells into the bloodstream. Proteins that are destined to be released from the cell travel through the endoplasmic reticulum and Golgi apparatus of pancreatic cells to the cell surface where they can be secreted.

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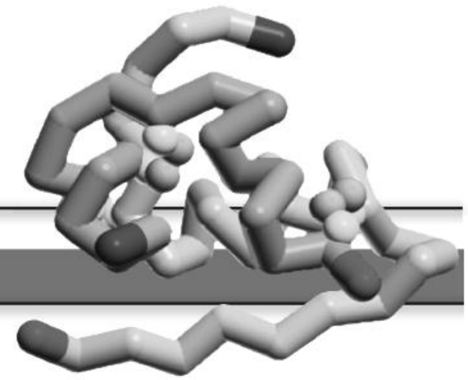


Preproinsulin - the Precursor Form of Insulin

Precursor Insulin

The precursor (inactive) form of insulin is known as *preproinsulin*. The first 24 amino acids of preproinsulin make up the Endoplasmic Reticulum (ER) Signal Sequence. As the protein is being synthesized, this signal sequence begins to emerge from the ribosome. Other proteins in the cell recognize this peptide and dock the ribosome onto the ER. As the rest of the protein is synthesized, it is directed through this membrane, into the lumen of the ER. From there, the preproinsulin is further processed (cleaved into four pieces) as it moves through the ER to the Golgi, and to the cell surface.

5. Locate, highlight and label the ER Signal Sequence on your Insulin Bioinformatics strip.

Signal Peptide

M A L W M R L L P L L A L L A L W G P D P A A A

a. Referring to the Standard Genetic Code table, categorize the chemical properties of each of the 24 amino acids that make up the ER Signal Peptide (hydrophobic, hydrophilic, positive charge, or negative charge). What is notable about the chemical properties of the amino acids that make up the ER Signal Peptide?

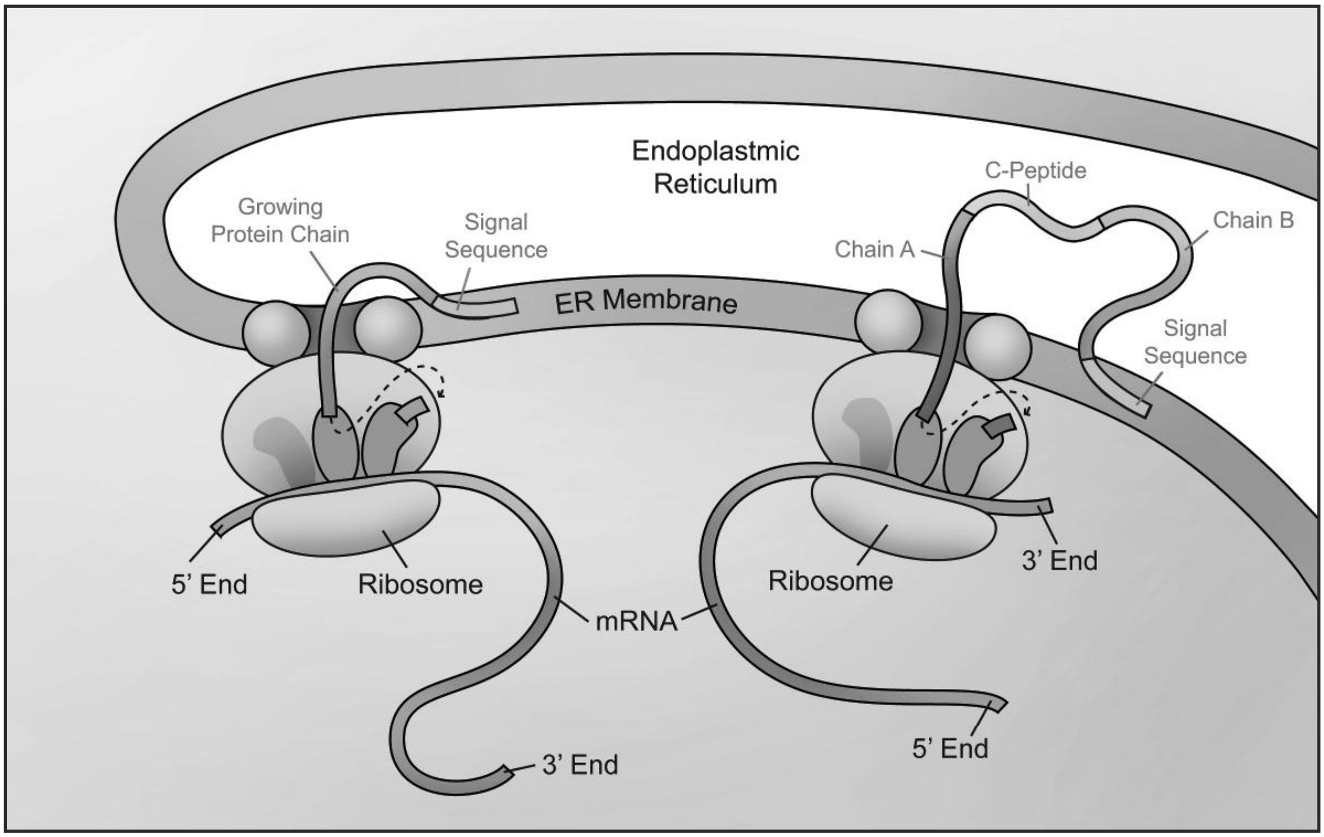
____

____

Preproinsulin to Proinsulin

Soon after the ribosome that is synthesizing preproinsulin is docked onto the ER, a protease in the ER cuts the precursor protein between amino acids 24 and 25 (Alanine, Ala, A and Phenylalanine, Phe, F). The 24 amino acid signal peptide is rapidly degraded, while the remaining 86 amino acid proinsulin begins its journey toward the Golgi and cell surface.

Proinsulin consists of the B-Chain (30 amino acids) and the A-Chain (21 amino acids), separated by the 35 amino-acid C-Peptide.



As proinsulin spontaneously folds into its final 3-D shape in the ER, another protease cuts the protein at two sites: between amino acids 54 and 55 (Threonine, Thr, T and Arginine, Arg, R) and between amino acids 89 and 90 (Agrinine, Arg, R and Glycine, Gly, G). As the C-Peptide is released from the folded B-Chain and A-Chain complex, it floats away and is degraded.

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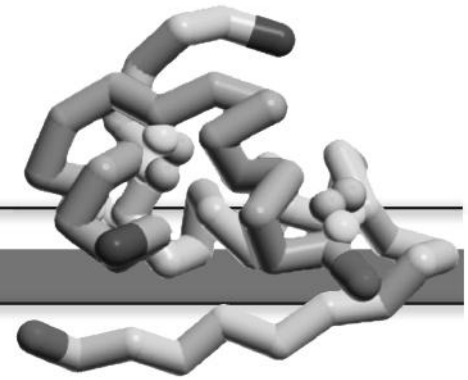
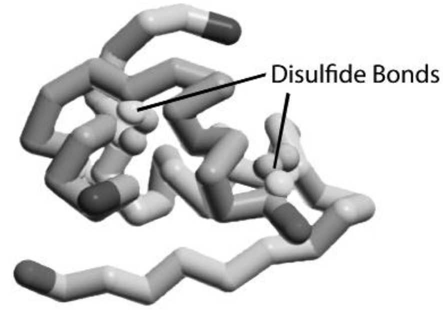


Preproinsulin to Proinsulin (continued)

6. Locate, highlight, and label the C-Peptide on your Insulin BioInformatics Map.



a. Since the C-Peptide is cut out of proinsulin to create the final mature insulin (Chain B and Chain A) what role do you think the C-Peptide might play in the biosynthesis of the mature insulin protein?

____

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As with many secreted proteins that must make their living in the harsh environment outside the cell, insulin is stabilized by two covalent disulfide bonds that join the B-Chain to the A-Chain. Each chain contributes one cysteine amino acid (Cys, C) to each disulfide bond.

Cys7 of the B-Chain forms a disulfide bond with Cys7 of the A-Chain.

Cys19 of the B-Chain forms a disulfide bond with Cys20 of the A-Chain.

A third disulfide bond forms between Cys6 and Cys11, both from the A-Chain.

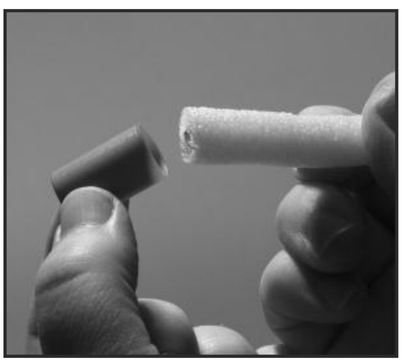
7. Circle each Cys on your Insulin mRNA to Protein map that participates in disulfide bond formation, and connect (with a line) the pairs that interact to form each disulfide bond.

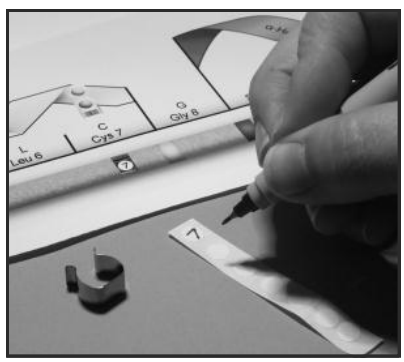
Folding the Physical Model of Insulin

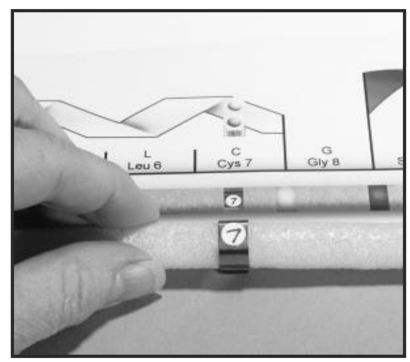
Like all proteins, insulin folds into a specific 3-D shape, following basic principles of chemistry. It is this 3-D shape that allows it to bind to the insulin receptor protein on the surface of liver, muscle, and fat cells to trigger the uptake of glucose from the bloodstream. In this final activity, you will shape two Mini-Toobers into the 3-D shape of the insulin protein.

1. Gather all of the parts you need:
   - Insulin Mini-Toober Folding Map
   - Orange and Purple Mini-Toobers
   - Bag with parts for Mini-Toobers
     - Sidechains
     - Metal Clips
     - Plastic Markers
     - Support Posts
     - White Dots
     - Endcaps

As you proceed with the directions (2) through (6) below you can work with the two chains at the same time or you can complete the B-Chain (orange Mini-Toober) and then repeat with the A-Chain (purple Mini-Toober).





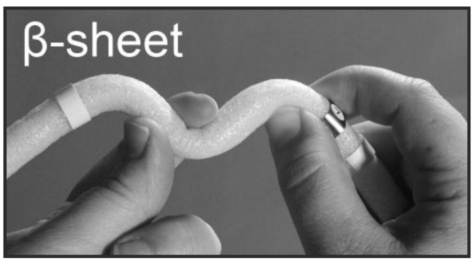


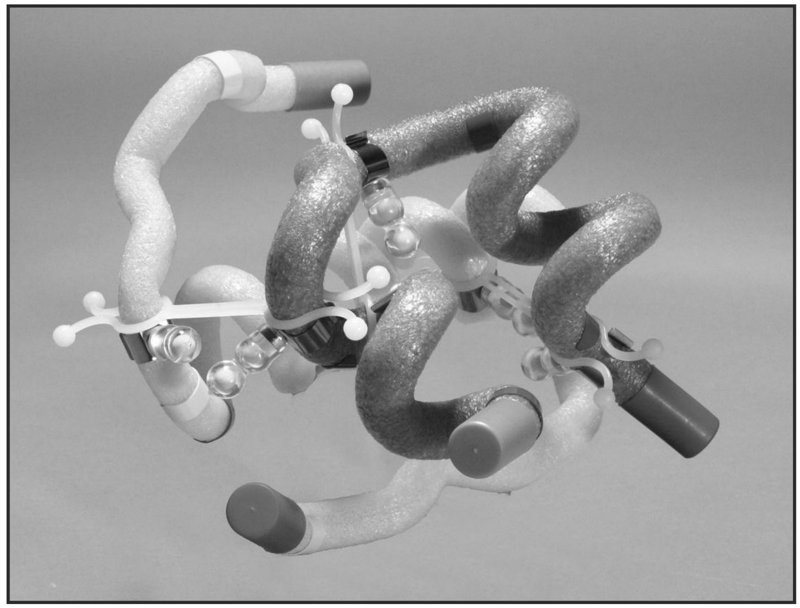
2. Unroll your Insulin Mini-Toober Folding Map and identify the **N-terminus (blue)** and the **C-terminus (red)** of each protein chain by putting one red and one blue end cap onto the ends of each Mini-Toober.

3. Using the Map, locate the cysteine amino acids on each protein chain. Write the number of each of the six cysteines on the white dots and add these numbered dots to six metal clips.

3. Carefully align each Mini-Toober with the corresponding chain on the Insulin Mini-Toober Folding Map matching the end caps to the images of the end caps on the Map. Add the appropriately numbered metal clips to the Mini-Toober. The metal clips represent the alpha-carbon of each cysteine amino acid.

Folding the Physical Model Of Insulin (continued)





4. Indicate where the α-helicies are on each protein chain by placing the red plastic markers at the beginning and the end of each α-helix. Indicate where the β-sheets are on each protein chain – by placing the yellow plastic markers on the Mini-Toober at the beginning and the end of each β-sheet shown on the Map.

5. Fold the Mini-Toobers to create:

   a. The α-helicies (right-handed) and the β-sheet strands (extended zig-zag) in each protein chain, and then

   b. The overall 3-D shape of each protein chain. Use the Jmol visualization tool and/or the images at the end of the Map to fold your insulin

6. Add the Cysteine sidechains to each of the six Cysteine alpha-carbon atoms.

7. Assemble the two chains into the final insulin model by positioning the chains as shown in the photo above, using the images on the map and/or the Jmol visualization tool located on the CD.

Note: The three pairs of Cysteine amino acids that form covalent disulfide bonds should be close to each other in the final model. Use the three plastic support posts to stabilize the protein at these three disulfide bonds.

Insulin In Review

• The insulin gene is located on the short arm of chromosome 11 in humans.

• The insulin gene is transcribed into an insulin mRNA molecule in the nucleus of the beta islet cells of the pancreas.

• The insulin mRNA is then transported to the cytoplasm of the cell where a ribosome recognizes the first AUG near the 5'-end of the mRNA and begins translating the protein, starting with methionine.

• The ribosome synthesizes a precursor form of insulin, known as preproinsulin.

• Preproinsulin is processed to become mature, functional insulin as it proceeds through the endoplasmic reticulum and Golgi apparatus, moving toward the cell membrane where it can be secreted from the cell.

• When there are high levels of sugar in the blood, insulin is released from the beta cells. It binds to receptors on the surface of liver, muscle, and fat cells. Binding of insulin results in a series of reactions within the cell (a signal cascade), leading to the fusion of vesicles containing glucose transporter proteins (GLUTS) with the membrane. The GLUTS transport glucose into the cells, where it is stored.